

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, including listings, of claims in the application.

Listing of Claims

Claim 1 (currently amended): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the *Pinus* subgenus with *Agrobacterium* for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection by washing cells with a liquid wash medium, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

2 (previously presented): The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;

(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

3 (previously presented): The method of claim 2, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

4 (previously presented): The method of claim 1, wherein said damage to cells is minimized by:

- (a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
- (b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

5 (previously presented): The method of claim 4, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

6 (previously presented): The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

Claim 7 (original): The method of claim 4, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

Claim 8 (original): The method of claim 7, wherein said steps are repeated between 2 and 10 times.

9 (previously presented): The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

Claim 10 (canceled).

Claim 11 (original): The method of claim 4, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 12 (previously presented): The method of claim 1, wherein said selection is performed by
culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

Claim 13 (original): The method of claim 12, wherein said selection agent is contained in said gel medium.

Claim 14 (original): The method of claim 12, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is placed on said gel medium.

Claim 15 (previously presented): The method of claim 14, wherein said layer is a layer of liquid medium.

Claim 16 (previously presented): The method of claim 14, wherein said layer is a layer of gelled medium.

Claim 17 (original): The method of claim 14, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 18 (original): The method of claim 12, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 19 (previously presented): The method of claim 1 which further comprises the eradication of *Agrobacterium* from the pine cells after incubation with *Agrobacterium*.

Claim 20 (previously presented): The method of claim 19, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 21 (previously presented): The method of claim 20, wherein said layer is a layer of liquid medium.

Claim 22 (previously presented): The method of claim 20, wherein said layer is a layer of gelled medium.

Claim 23 (original): The method of claim 20, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 24 (original): The method of claim 20, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 25 (currently amended): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the subgenus *Pinus* with *Agrobacterium* for *Agrobacterium* transformation;

eradicating *Agrobacterium* from the pine cells after incubation with *Agrobacterium*;

minimizing damage to cells subsequent to *Agrobacterium* infection by washing cells with a liquid wash medium, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;
selecting transformed cells;
culturing said transformed cells to produce transgenic somatic embryos; and
germinating said transgenic somatic embryos to produce transgenic plants.

Claim 26 (previously presented): The method of claim 25, wherein said damage to cells is minimized by:

- (a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;
- (b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 27 (previously presented): The method of claim 26, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 28 (previously presented): The method of claim 26, wherein said selection is performed by

- culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
- contacting said cells with a selection agent; and
- selecting transformed cells.

Claim 29 (previously presented): The method of claim 26, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 30 (previously presented): The method of claim 28, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 31 (previously presented): The method of claim 25, wherein said damage to cells is minimized by:

- (a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
- (b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 32 (previously presented): The method of claim 31, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 33 (previously presented): The method of claim 31, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

Claim 34 (previously presented): The method of claim 31, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and

selecting transformed cells.

Claim 35 (previously presented): The method of claim 31, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 36 (previously presented): The method of claim 34, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 37 (previously presented): The method of claim 25, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

Claim 38 (previously presented): The method of claim 25, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 39 (previously presented): A method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation which comprises:

- (a) washing transformed cells of the subgenus *Pinus* in a liquid wash medium;
- (b) plating said cells on a support membrane;
- (c) suspending said cells in a liquid wash medium; and
- (d) recovering washed cells with minimal physical damage.

Claim 40 (original): The method of claim 39, wherein (i) cells are plated onto a support membrane and (ii) said cells are transformed prior to step (a).

Claim 41 (original): The method of claim 39, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

Claim 42 (original): The method of claim 41, wherein said steps are repeated between 2 and 10 times.

Claim 43 (previously presented): The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

Claim 44 (canceled).

Claim 45 (original): The method of claim 39, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 46 (previously presented): A method for pine cell tissue culture which comprises culturing pine cells of the genus *Pinus* subgenus *Pinus* on a support membrane placed over a gel medium.

Claim 47 (previously presented): The method of claim 46, wherein said support membrane is placed over a layer containing one or more tissue culture medium constituents, said layer is positioned on said gel medium.

Claim 48 (original): The method of claim 46, wherein said cells are plated onto said support membrane prior to culturing.

Claim 49 (previously presented): The method of claim 47, wherein said layer is a layer of liquid medium.

Claim 50 (original): The method of claim 47, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 51 (original): The method of claim 46, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 52 (previously presented): A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus subsequent to transformation on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

Claim 53 (original): The method of claim 52, wherein said selection agent is contained in said gel medium.

Claim 54 (original): The method of claim 52, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is positioned on said gel medium.

Claim 55 (previously presented): The method of claim 54, wherein said layer is a layer of liquid medium.

Claim 56 (original): The method of claim 54, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 57 (original): The method of claim 52, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer.

Claim 58 (previously presented): A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus on a support membrane over a layer containing an eradicator, said layer positioned in or over a gel medium; and

recovering cells from which said *Agrobacterium* contaminant has been eradicated.

Claim 59 (previously presented): The method of claim 58, wherein said layer is a layer of liquid medium.

Claim 60 (previously presented): The method of claim 58, wherein said layer is a layer of gelled medium.

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Claim 61 (original): The method of claim 58, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 62 (original): The method of claim 58, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claims 63-81 (canceled).